cell-attached patch recording is made with an electrode containing nystatin. The nystatin pores permit electrical continuity with the cell but do not allow exchange of larger substances (for example, ATP) between the pipette and the cell. We monitored calcium currents to ensure that the nystatin patch did not rupture during the recording.

10. A glass pipette (outer diameter, 0.75 mm) was inserted into the tip of the patch electrode (perfusion rate, 0.01 to 0.02 ml/min).
11. Adenosine 3',5'-monophosphothioate (cAMPS) is an analog of cAMP that includes a sulfur in the phosphate moiety, and the phosphate can exist in one of two (*R* and *S*) diastereomeric forms (Sp-cAMPS and Rp-cAMPS; BioLog Life Science Institute, Bremen, Federal Republic of Germany). The Sp isomer is an agonist of cAMP-dependent protein kinases type I and II, and the Rp isomer is a specific inhibitor [T. Braumann and B. Jastorff, *J. Chromatog.* **350**, 105 (1985); B. Yusta *et al.*, *J. Neurochem.* **51**, 1808 (1988)].
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Enhancement of the Glutamate Response by cAMP-Dependent Protein Kinase in Hippocampal Neurons

PAUL GREENGARD, JOANNA JEN, ANGUS C. NAIRN, CHARLES F. STEVENS*

Receptor channels activated by glutamate, an excitatory neurotransmitter in the mammalian brain, are involved in processes such as long-term potentiation and excitotoxicity. Studies of glutamate receptor channels expressed in cultured hippocampal pyramidal neurons reveal that these channels are subject to neuromodulatory regulation through the adenylate cyclase cascade. The whole-cell current response to glutamate and kainate [a non-NMDA (*N*-methyl-D-aspartate) receptor agonist] was enhanced by forskolin, an activator of adenylate cyclase. Single-channel analysis revealed that an adenosine 3',5'-monophosphate–dependent protein kinase (PKA) increases the opening frequency and the mean open time of the non-NMDA–type glutamate receptor channels. Analysis of synaptic events indicated that forskolin, acting through PKA, increased the amplitude and decay time of spontaneous excitatory postsynaptic currents.

BRAIN SECOND-MESSENGER SYSTEMS often alter nerve cell activity by modifying characteristics of voltage-gated channels (*1–3*). Although the regulation of neuronal excitability by phosphorylation of voltage-gated channels is widespread, the neuromodulation of ligand-gated channels, crucial for the control of synaptic strength, has been reported in only a few instances (*4–6*). Because the modification of synaptic strength through the action of second-messenger systems should be an effective way to regulate the computational capabilities of neuronal circuits, this form of neuromodulation could be as common as the ones that affect electrical excitability. We anticipated that the hippocampus might be a favorable site to search for synaptic neuromodulation; this brain region is well known to regulate synaptic strength by long-term potentiation (*7*), and the abundantly represented glutamate receptor channels in hippocampus have been implicated in memory, epilepsy, excitotoxicity, and some neurodegenerative diseases (*8*).

We used both the whole-cell recording and the outside-out mode of the standard patch-clamp method (*9*) to study responses to glutamate in 2- to 7-day-old cultured hippocampal pyramidal neurons from newborn Long Evans rats (*10*). We included 5 μM magnesium adenosine trisphosphate (MgATP) in all the whole-cell recordings to prevent "run-down" of glutamate-induced responses (*11*).

Because adenylate cyclase is commonly involved in neuromodulatory systems, we examined responses to glutamate in whole-cell recordings before and after treatment with forskolin (FSK), an activator of adenylate cyclase (*12*). In the continual presence of 50 μM FSK, glutamate responses increased in all cells tested (+91% ± 27% at 15 min after glutamate application; $n = 4$; mean ± SEM) (Fig. 1). For comparison, we recorded the responses in another set of cells not treated with FSK. The responses to glutamate in this control group of cells generally decreased over the same time interval (−30% ± 10% at 15 min after glutamate application; $n = 4$) (Fig. 1B). Thus, FSK produces a marked neuromodulation of glutamate receptors.

FSK can sometimes interact directly with ion channels without the intermediary action of the adenosine 3',5'-monophosphate (cAMP) cascade (*13, 14*), and a similar direct mechanism might conceivably account for the FSK effect on glutamate receptor channels. In an effort to determine if FSK acts directly, we treated our cells with 1,9-dideoxyforskolin, a structural analog. Dideoxyforskolin does not stimulate adenylate cyclase but does mimic other actions of FSK. Of the three cells treated with 50 μM dideoxyforskolin, none showed an increased response to glutamate (−23% ± 10%; $n = 3$) (Fig. 1B).

If the increased glutamate response involves the cAMP-dependent protein kinase (PKA), then blocking kinase activity should prevent the neuromodulatory effect of FSK. To test this, we added IP_{20}-amide, a 20-amino acid synthetic peptide that specifically inhibits PKA (*15*), to the solution in the recording pipette. IP_{20}-amide (1 μM) in the electrode blocked the neuronal response to FSK (−28% ± 5%; $n = 6$) (Fig. 1B). In addition, with IP_{20}-amide alone in the recording pipette (and no FSK) the response to glutamate became smaller than the control within 20 min (−50% ± 9%; $n = 4$) (Fig. 1B), suggesting a basal PKA activity.

The increased macroscopic response to glutamate after FSK treatment could have resulted from changes in various biophysical properties of the glutamate channels, such as a more frequent entry into the open state, a lengthened stay in the open state, or an increased unitary conductance. Furthermore, glutamate and its structural analogs activate a mixture of receptor subtypes. *N*-methyl-D-aspartate (NMDA) preferentially activates large conductance (40 to 50 pS) channels, whereas non-NMDA channels—kainate, quisqualate, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid—preferentially activate small conductance (typically less than 30 pS) channels (*16*). The non-NMDA channels are difficult to distinguish from each other but are distinct from the NMDA class (*16, 17*). Changes in either or both types of channels could have led to an increased whole-cell current response to glutamate.

To determine the basis for the increased

P. Greengard and A. C. Nairn, Laboratory of Molecular and Cellular Neuroscience, Rockefeller University, New York, NY 10021.
J. Jen, Neuroscience Program, Yale University, New Haven, CT 06510, and Molecular Neurobiology Laboratory, Salk Institute, La Jolla, CA 92037.
C. F. Stevens, Molecular Neurobiology Laboratory, Salk Institute, La Jolla, CA 92037.

*To whom correspondence should be addressed.

macroscopic response, we carried out single-channel analysis on outside-out patches from the soma of hippocampal pyramidal neurons. Glutamate was used as the agonist because it probably is the natural neurotransmitter and activates all subtypes of receptors. The results are summarized in Table 1. The non-NMDA–type channels in membrane patches (n = 6) with the internal surface exposed to 20 g/ml of catalytic subunit of PKA (cPKA) in the presence of 5 μM MgATP showed more frequent and longer openings compared to control patches (n = 9). Inclusion of MgATP alone (n = 6), 20 g/ml of heat-denatured cPKA (dPKA) with MgATP (n = 6), or cPKA without MgATP (n = 4) yielded results similar to those obtained with control patches, with mean open times comparable to reported values (*16*) (Table 1 and Fig. 2).

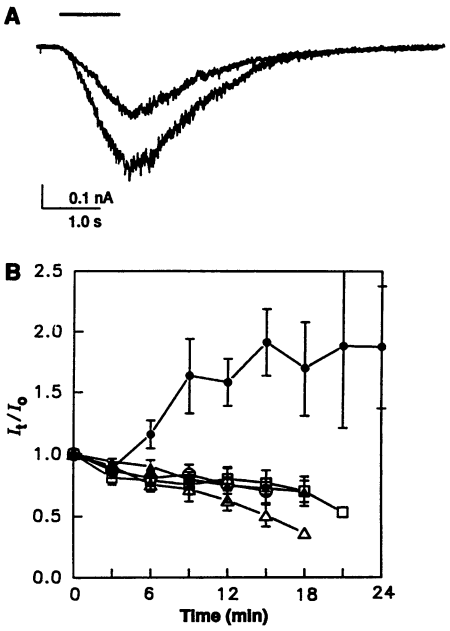

Fig. 1. (**A**) Whole-cell current response to the application of 10 μM glutamate at a holding potential of −60 mV. The upper trace was recorded before the neuron was perfused with FSK. The lower trace was recorded 6 min after the same cell was perfused with 50 μM of FSK. (**B**) The ratios of whole-cell glutamate current responses (I_t) at the indicated times to the response at the beginning of the experiment I_0. Data are presented as mean ± SEM; n = 3 to 6. (○), control; (●), FSK; (□), dideoxyforskolin; (△), IP_{20}-amide; (▲), IP_{20}-amide + FSK.

In contrast, the properties of the NMDA receptor channels were indistinguishable under all conditions. The opening frequency of NMDA receptor channels in membrane patches treated with cPKA plus MgATP was the same as that in control patches; further, the mean open time of NMDA-evoked events in cPKA plus MgATP–treated patches did not differ from the mean open time in control patches (Table 1). Neither the non-NMDA nor the NMDA receptor subtypes showed any change in unitary conductance.

The increase in the opening frequency of the non-NMDA receptor channels and their longer time in the open state might account for the larger macroscopic response to glutamate seen after FSK treatment. We thus used whole-cell recording to examine whether FSK increased the current response to kainate, an agonist selective for non-NMDA–class channels (*16*). Indeed, the response to kainate increased in the presence of FSK (+80% ± 44% 15 min after kainate application; n = 7; ± SEM) compared with the response in control cells (−30% ± 6%; n = 9), cells treated with dideoxyforskolin (−22% ± 24%; n = 2), or cells treated with IP_{20}-amide plus FSK (−32% ± 10%; n = 3) (Fig. 3). This increase in the kainate response with FSK is comparable to the increase in the glutamate response. Thus, the neuromodulation of the non-NMDA class of glutamate receptor channels can account for much, if not all, of the FSK effect on whole-cell responses to glutamate.

We have thus far described the effects of the cAMP second-messenger system on extrajunctional glutamate channels, rather than on those localized in the postsynaptic membrane. Studies of the acetylcholine receptors in muscle reveal marked differences in ion channel properties between junctional and extrajunctional receptors (*18*). To determine if junctional glutamate channels might also be modulaed through the cAMP cascade, we recorded miniature excitatory postsynaptic currents (MEPSCs) under whole-cell voltage clamp (*19*). MEPSCs recorded in the presence of FSK increased in amplitude, decay time, or both, as compared to recordings made before FSK treatment (Fig. 4). Of the 11 cells treated with FSK, MEPSCs in seven increased in amplitude (46% ± 20%; ± SEM), and six increased in decay time (58% ± 32%); in five of the 11 cells, both the amplitude and the decay time of the MEPSCs increased (P < 0.05, paired t test). Because the spontaneous MEPSCs recorded from cells under different control conditions [untreated (n = 8); dideoxyforskolin (n = 4); IP_{20}-amide (n = 2); IP_{20}-amide plus FSK (n = 2)] showed little change in amplitude or decay time, we conclude that PKA activity probably accounts for the effect of FSK on the MEPSCs. The frequency of the MEPSCs also increased with FSK treatment, suggesting that cAMP-dependent protein phosphorylation might increase the probability of release of vesicles presynaptically in addition to enhancing the glutamate response postsynaptically.

Our results indicate that the properties of

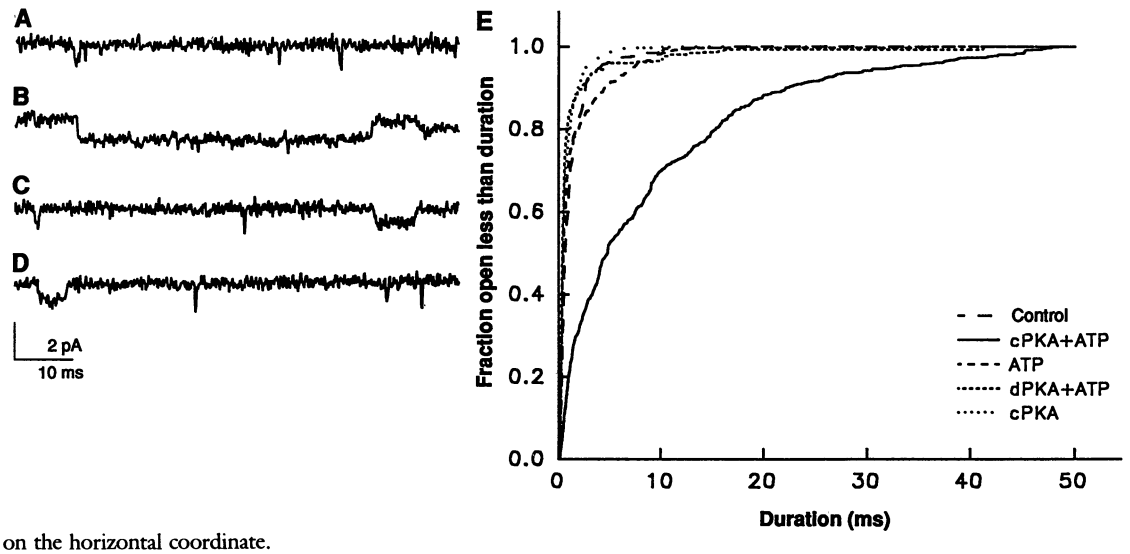

Fig. 2. (**A** through **D**) Examples of single-channel recordings of non-NMDA–type activity from four outside-out patches held at −110 mV with different reagents in the recording electrodes: (A) control; (B) cPKA (20 μg/ml) plus 5 mM MgATP; (C) 5 mM MgATP alone; and (D) dPKA (20 μg/ml) plus 5 mM MgATP. (**E**) Cumulative open-time graphs of non-NMDA channels under the indicated conditions. All data were taken from outside-out patches held at −110 mV. The vertical coordinate represents the fraction of the channels that stayed open for less than the time duration that is indicated on the horizontal coordinate.

Table 1. Comparison of opening frequencies and mean open times of glutamate receptor channels under different conditions. All data are presented as mean ± SEM and were obtained from patches held at −110 mV. Events, the number of events per second. ATP, adenosine triphosphate; *n*, number of patches.

Condition (*n*)	Non-NMDA responses		NMDA responses	
	Events	Mean open time (ms)	Events	Mean open time (ms)
Control (9)	0.83 ± 0.28	1.40 ± 0.14	7.63 ± 1.87	2.61 ± 0.49
ATP (6)	0.95 ± 0.29	1.65 ± 0.43	7.73 ± 2.12	2.32 ± 0.50
dPKA + ATP (6)	0.42 ± 0.10	1.39 ± 0.43	10.8 ± 1.88	2.80 ± 0.21
cPKA (4)	1.10 ± 0.17	0.92 ± 0.28	7.70 ± 2.12	3.31 ± 0.37
cPKA + ATP (6)	2.42 ± 0.73*	10.3 ± 1.72**	8.34 ± 1.31	1.94 ± 0.34

*$P < 0.05$, one-way analysis of variance. **$P < 0.001$, one-way analysis of variance.

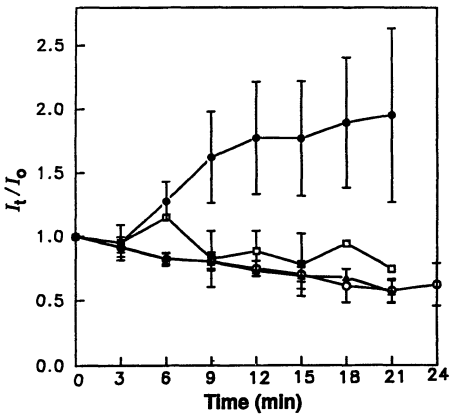

Fig. 3. The ratios of whole-cell current responses to kainate (I_t) at the indicated times to the response at the beginning of the experiment I_0. Data are presented as mean ± SEM; n = 2 to 9. (○), Control; (●), FSK; (▲), FSK + IP_{20}-amide; (□), dideoxyforskolin.

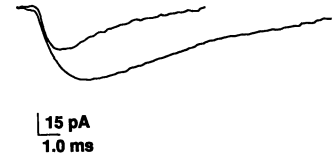

Fig. 4. Average traces of spontaneous MEPSCs recorded at −60 mV before and after treatment with FSK. The average of 30 MEPSCs before (upper trace) and after (lower trace) constant perfusion of the same neuron with 50 μM FSK.

both junctional and extrajunctional glutamate receptor channels are regulated by PKA. The single-channel recording experiments suggest either that the channels themselves are phosphorylated directly or that regulatory proteins necessary for modulation of glutamate channels are present in the membrane and are phosphorylated. Whether the apparent increase in the opening rate of non-NMDA channels resulted from an increased opening probability of channels or an increased number of channels could not be determined with our methods.

The same modulatory machinery could function in synaptic junctions as well as extrajunctionally. Our single-channel recordings revealed an increase in the opening frequency and the mean open time of the non-NMDA channels. These channels have been reported to mediate fast excitatory synaptic transmission (*20*). An increase in the opening frequency and the mean open time of the junctional non-NMDA channels could account for the increase in the amplitude or decay time of the spontaneous MEPSCs observed with FSK treatment.

Our experiments do not exclude possible modulation of NMDA receptor channels. Both NMDA and non-NMDA receptors could have contributed to the increased whole-cell current response to glutamate observed with FSK treatment, but the fact that FSK treatment enhanced the kainate responses and the glutamate responses at comparable amounts suggests that the NMDA channels contribute little to the process. In addition, single-channel analysis under various conditions revealed no obvious alteration of NMDA channel characteristics, demonstrating that modulation of NMDA channels by PKA is absent or at least less prominent than modulation of non-NMDA receptor channels. Neuromodulation of NMDA response has been proposed (*21*), but only further investigation can determine whether NMDA channels can be modulated by the cAMP cascade or other signal transduction systems.

The regulation by phosphorylation of glutamate channel activity provides a potentially important way for the nervous system to control the excitability of neurons and the efficacy of synaptic transmission. We anticipate that investigation will reveal that synapses in many brain regions are subject to neuromodulatory control, just as are many types of voltage-gated channels.

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10. We dissected hippocampi from newborn rats and triturated the tissue to yield dissociated cells that were plated onto collagen–poly-D-lysine–coated glass coverslips and maintained in minimum essential medium supplemented with 2% glucose, 5% fetal bovine serum, and 0.1% serum extender in an incubator until ready for recording 2 to 7 days later. The external solution was composed of 160 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 10 mM Hepes, 1 μM glycine, 1 μM tetrodotoxin, and 100 μM picrotoxin. The internal solution was composed of 140 mM Cs methanesulfonate, 5 mM CsCl, 5 mM Hepes, and 10 mM EGTA or Cs-bis(*o*-aminophenoxy)ethane *N,N,N',N'*-tetraacetic acid. All salts were purchased from Alfa (Puratronic grade, Ward Hill, MA), and solutions were adjusted to pH 7.4. Electrodes had a resistance of 2 to 3 megohms for whole-cell recordings and 3 to 4 megohms for single-channel recordings. All recordings were performed with an Axopatch 1B amplifier (Axon Instruments, Foster City, CA). Data were acquired on-line and signals were digitized through Labmaster (Scientific Solutions). For whole-cell recordings, a blunt-ended pipette with glutamate in the external solution was placed 30 μm from the tip of the recording pipette. Driven by air pressure, the puffer pipette released agonist to elicit current response. Neuronal current responses to agonist puffs were sampled at 2-ms intervals and filtered at 500 Hz. FSK and dideoxyforskolin were continuously applied to the bath in separate experiments at a final working concentration of 50 μM. IP_{20}-amide was used at 1 mM. For single-channel recordings, we used outside-out patches because the amount of the agonist and the duration of the agonist application were easily controlled in this mode, which is appropriate for the study of agonist-activated channels. We included cPKA (20 μg/ml) in the recording pipette where indicated. We boiled an aliquot of cPKA for 30 min to 1 hour to denature the enzyme and produce dPKA. Data were sampled at 100-μs intervals and filtered at 2000 Hz. Conductance levels were set visually at 40 pS for NMDA channels and 10 pS for non-NMDA channels. Glutamate was applied at such a low concentration and duration that simultaneous openings were rarely observed. All transitions exceeding 50% of the preset conductance level were confirmed. Channel openings that lasted for less than 300 μs were not included in the analysis. The open times referred to the durations between transitions; true closures and brief interruptions of open-channel current were not distinguished in this analysis. Initially, we found in several experiments that the mathematical averages of groups of open times approximated the mean open times obtained from curve-fitting raw data. Thereafter, the mathematical averages were calculated.
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Anti-HIV and Anti–Anti-MHC Antibodies in Alloimmune and Autoimmune Mice

TRACY A. KION AND GEOFFREY W. HOFFMANN*

Alloimmune mice (mice that have been exposed to cells from another murine strain) were shown to make antibodies against gp120 and p24 of human immunodeficiency virus (HIV), and mice of the autoimmune strains MRL-*lpr/lpr* and MRL-+/+ made antibodies against gp120. This is surprising because the mice were not exposed to HIV. Furthermore, anti–anti-MHC antibodies (molecules that have shapes similar to those of major histocompatibility complex molecules) were detected in both alloimmune sera and MRL mice. These results are discussed in the context of a possible role for allogeneic stimuli in the pathogenesis of acquired immunodeficiency syndrome, as suggested by an idiotypic network model.

THE FUNCTIONAL DISRUPTION OF the immune system in acquired immunodeficiency syndrome (AIDS) is incompletely understood and may involve autoimmunity (*1*). Shearer suggested a possible role for allogeneic cells (cells from another individual) in AIDS pathogenesis on the basis of similarities between AIDS and graft-versus-host disease (*2*). Exposure to allogeneic cells can occur when individuals are exposed to foreign lymphocytes in blood transfusions or ejaculates. Ziegler and Stites (*3*) and Andrieu and co-workers (*4*) formulated an idiotypic network model for HIV in which it is suggested that gp120 of HIV may cross-react with class II MHC molecules, and an anti-idiotypic component of an immune response to gp120 could cross-react with the CD4 molecules on helper T cells. Such ideas are supported by the findings that large quantities of antigen-mimicking antibodies can be produced in ordinary immune responses (*5*), and many antibodies have both anti-idiotypic and antigen-specific activity (*6*). We formulated an autoimmunity model of AIDS pathogenesis that involves two immune responses, namely the immune response to HIV and an immune response to allogeneic stimuli (*7*). The two responses include components that are directed against each other, and these responses are postulated to synergize in a way that causes the collapse of the immune system.

Two separable classes of specific antibodies can be detected in alloimmune sera, namely anti-foreign and anti–anti-self (*8*). Anti–anti-self antibodies are made in response to foreign idiotypes that recognize self MHC and may have MHC-image (MI) activity, as determined by their ability to completely inhibit cytotoxicity mediated by polyclonal alloantisera (*8*). This component of immunity to allogeneic lymphocytes may be related to immunity induced to HIV, because the envelope protein of HIV (gp160) has a shape that may be partly MI. One of its components (gp120) binds to CD4 at a site that overlaps with the site where CD4 interacts with class II MHC (*9, 10*), and there is also cross-reactivity between the other component (gp41) and class II MHC (*11*). Furthermore, sequence similarities have been observed between gp120 and class II MHC (*12*) and between HIV-1 Nef and class II MHC (*13*). Because HIV components may have shapes that are partly MI, it is plausible that HIV provokes an immune response that includes an anti–class II MI (anti-MI) component. MI and anti-MI responses are by definition directed against each other, so MI and anti-MI lymphocytes could be stimulated by each other. Hence, an MI response to allogeneic cells could synergize with an anti-MI response to HIV (*7*).

Helper T cells have variable (V) regions that are selected to be weakly anti–self class II MHC. We have described an idiotypic network model in which suppressor T cells recognize and regulate helper T cells by means of their V regions; these T cells have receptors with class II MI determinants (*14*). The MI response to allogeneic cells and the anti-MI response to HIV could also be directed against anti-MHC idiotypic determinants of helper T cells and MI idiotypic determinants on suppressor T cells, respectively, and this dual attack could lead to the eventual collapse of the normal self-stabilizing system.

We considered the possibility that the image of MHC might be conserved across species, even though CD4, which has complementarity to class II MHC is not conserved between mice and humans with respect to its ability to bind gp120 of HIV. HIV gp120 binds to human CD4 but not mouse CD4. If the MI is at least partly conserved, alloimmune mice could make anti-MI antibodies that react with gp120. We raised alloimmune sera in pairs of strains of mice by repeated reciprocal immunizations with lymphoid cells. In agreement with previous work (*8*), the alloimmune sera were found to contain MI antibodies (*15*). These sera also contain antibodies to gp120 and p24 (Fig. 1, A and B). Similar results were obtained with all eight hyperimmune

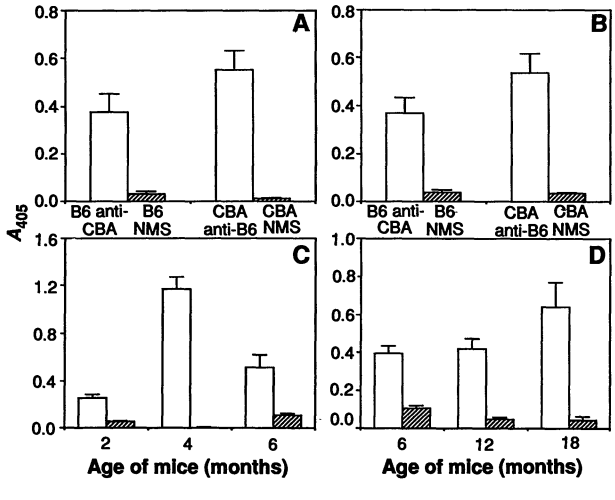

Fig. 1. Antibodies to gp120 (**A**) and p24 (**B**) were detected in B6 anti-CBA and CBA anti-B6 alloantisera, but not in normal (NMS) CBA or B6 sera. Antibodies to gp120 were also detected in the sera of MRL-*lpr/lpr* mice (**C**) (white bars) and in MRL-+/+ mice (**D**) (white bars) but not in age-matched CBA controls (hatched bars) (*17*). A_{405}, absorbance at 405 nm.

T. A. Kion, Department of Microbiology, University of British Columbia, Vancouver, BC, Canada V6T 1W5.
G. W. Hoffmann, Departments of Microbiology and Physics, University of British Columbia, Vancouver, BC, Canada, V6T 1W5.

*To whom correspondence should be addressed.